Symposium: Large Complexes and Machines - Dissecting Mechanism

2171-Symp
Mechanism and Function of the Eukaryotic Ring-Shaped Chaperonin TRiC/CCT
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Protein folding in the cell is critically dependent on the assistance of molecular chaperones. The chaperonin of eukaryotic cells, TRiC, is a 1 MDa ring-shaped complex that folds many essential cellular proteins. TRiC is hetero-oligomeric and uses ATP binding and hydrolysis to open and close a built-in lid over the central cavity. Notably, TRiC has the ability to fold some eukaryotic proteins, such as actin, that cannot be folded by any other chaperone. Thus understanding TRiC mechanism is an integral first step to deciphering the role of this chaperonin in cellular folding and potentially ameliorating human misfolding disorders. Recent insights into the structural and mechanistic basis of the conformational cycle of TRiC will be discussed, as well as their implications for polypeptide-mediated folding.

2172-Symp
Conformational Dynamics, Structure & Substrate Protein Interactions of Hsp90 Chaperones
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Hsp90 is generally thought to transition between an open, apo and closed, 'ATP' conformation. A third, ADP state has been observed with the E. coli Hsp90. Using a combination of SAXS, EM single particle and crosslinking methods, we find that that all three states are universally conserved between bacteria, yeast and human Hsp90s. However the equilibrium and kinetics of the transitions between sates differ significantly. To increase conformational diversity in the E coli chaperone, there are two apo conformations (an open and Grp94-like state) established by a pH-dependent titration of a specific histidine. We also show that the conformational states are functionally distinct. These data support a conserved multi-state chaperone cycle where the conformational equilibrium varies between species, implicating evolutionary tuning of the different states to meet the specific client protein and metabolic environment of an organism. Current efforts focus on client-Hsp90 and cochaperone-Hsp90 interactions. We show that a deletion mutant of staph nuclease (Δ131Δ) that is unfolded and non-aggregating provides a powerful model substrate for interrogating Hsp90-client interactions. NMR reveals a distinct ~25 residue region (the most structured within Δ131Δ) that interacts strongly with Hsp90, resulting in dramatic shifts in Hsp90 conformation. SAXS and NMR identify a region of the Hsp90 MD as the primary site of Δ131Δ binding. Most importantly, FRET measurements demonstrate that this model substrate catalyzes Hsp90 closure in the presence of AMPPNP. Similar effects have now been observed for other clients and with other Hsp90s. The human mitochondrial Hsp90 TRAP1 has a remarkable temperature-dependent closure demonstrating that kinetic rather than thermodynamic control is critical for understanding Hsp90 function. Three-dimensional cryoEM reconstructions for Hop-Hsp90, Hsp70-Hop-Hsp90complexes are underway and will be discussed.

2173-Symp
Unexpected Sophistication and Complexity of Chemomechanical Control Linkages in Nucleic Acid Motor
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Ring-shaped, oligomeric ATPases are essential for a variety of cellular processes ranging from protein and nucleic acid metabolism to organelle transport. A subset of these motor proteins, the hexameric helicases, couple the binding and hydrolysis of ATP to the physical manipulation of nucleic acids to support essential cellular processes such as gene regulation, DNA replication, and DNA repair. How nucleotide turnover is coordinated between six independent motor subunits to generate helicase movement has been a long-standing question in the field. The topological problem of how extended nucleic substrates are loaded into the central pore of a closed hexameric toroid is similarly not well-understood. Using the 300 kDa E. coli Rho protein as a model hexameric RNA/DNA helicase, we have examined these issues through a combined structural and biochemical approach. Models derived from high-resolution crystallographic and solution X-ray scattering data explain in molecular detail how the Rho ring opens and closes in response to substrate binding, how the ATP cycle is coordinated with RNA movement through the motor interior, and why certain helicase families translocate with a preferred directional polarity. We find an unexpectedly rich array of physical connectors that sense the RNA binding status of individual subunits in the motor, and that appear to couple these states to the timing of ATP hydrolysis in a manner that is responsive to the base sequence of the substrate. These studies highlight the physical complexity of hexameric helicases, and suggest that related motor proteins utilize similarly intricate chemomechanical linkage mechanisms.

2174-Symp
Mechanisms of Chromatin Remodeling Motors
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ATP-dependent chromatin remodeling motors regulate the access of DNA during replication, transcription, recombination and repair. The mechanisms by which these motors function are not well understood. We will present our recent advances on understanding the mechanism of the chromatin remodeling complex, ACF.

Platform: Micro & Nanotechnology: Nanopores II

2175-Plat
New Technique of DNA Sensing: Nanoribbon Transverse Electrodes
Vita Solovyeva[1,2], Edmond Chow[2], Myung-Ho Bae[1,2], David Estrada[1,2], Shouvik Banerjee[2,3], Ashkan Behnam[1,2], Vincent E. Dorgan[1,2], Woo-Jin Chang[1,2], Eric Pop[1,2], Rashid Bashir[1,2].
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The technique of DNA sensing by electrophoretically driving it through solid-state nanopores is promising to reach the goal of rapid sequencing of individual human genomes. This approach can allow for the label-free, amplification-free analysis of nucleic acids as either single stranded DNA or double stranded DNA. Molecules ranging in length from single nucleotides to kilobase-pair can potentially be analyzed with nanometerresolution.
However, the standard 2-terminal nanopore sensing method also possesses disadvantages, as the measurement resolution does not allow sensing of individual nucleobases. In order to eliminate this disadvantage, modification of the nanopore sensing technique has been proposed theoretically [1, 2]. We are pursuing the structure of a graphene nanoribbon with an embedded nanopore as a transverse electrode. A unique change of the transverse current for each nucleobase could allow discrimination between different types of nucleotides.
The graphene nanoribbons are fabricated on a suspended stacked graphene/dielectric nanocomposite membrane. The stacked membrane was implemented according to the method described recently [3]. The top graphene layer was patterned by e-beam lithography in a 2 μm long x 30 nm wide nanoribbon. We will report on the transport measurements in air and in buffer solution with and without the embedded nanopore within the graphene nanoribbon.
[1] T. Nelson, B. Zhang, and O.V. Prezhdo, *Nano Lett*. **10**, 3237 (2010).
[2] K.K. Saha, M. Drndic, B.K. Nikolic, arXiv:1108.3801v1 (2011).
[3] B. M. Venkatesan, D. Estrada, S. Banerjee, X. Jin, V. E. Dorgan, M.-H. Bae, N. Aluru, E. Pop, and R. Bashir, submitted, (2011).

2176-Plat
High-Bandwidth Solid-State Nanopore Sensors
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Nanopores are attractive electrochemical sensors for their single-molecule sensitivity and simple experimental setup. Many of the electronic systems used for nanopore measurements are inherited from electrophysiology applications, and as a result they are primarily optimized for current levels of 50pA or less, at bandwidths below 10kHz. However, state-of-the-art solid-state nanopore sensors now commonly produce signals in the nanoampere range, for which common patch clamp amplifiers are no longer ideal. We present a new experimental platform which integrates a custom CMOS preamplifier with nanopores in thin silicon nitride membranes. The low parasitic capacitance of the integrated platform reduces noise at high frequencies, allowing nanopore measurements at finer temporal resolution than supported by popular instruments. We demonstrate the viability of this system with high-bandwidth electrical recordings of the passage of short DNA molecules through nanopores. This new platform will improve the signal-to-noise ratios of nanopore sensors, enabling further single-molecule studies of fast biophysical processes.